CLAIMS

1. A fluorescent indicator, which has a structure such that a donor fluorescent protein and an acceptor fluorescent protein bind to both termini of the target sequence of an analytical substance, wherein the analytical substance binds to or acts on said target sequence, so that the three-dimensional structure of the indicator can be changed, thereby generating fluorescence resonance energy transfer (FRET), said fluorescent indicator being characterized in that the said donor fluorescent protein and/or said acceptor fluorescent protein are circularly permuted fluorescent proteins obtained by substituting the amino acid sequence on the N-terminal side of a wild-type fluorescent protein or a mutant protein thereof with the amino acid sequence on the C-terminal side thereof, and in that the thus obtained fluorescent proteins have a fluorescent peak wavelength substantially identical to that of a florescent protein, which has not yet been subjected to the circular permutation.

2. The fluorescent indicator according to claim 1 wherein the fluorescent protein is GFP, CFP, YFP, RFP, BFP, or a mutant thereof.

3. The fluorescent indicator according to claim 1 or 2 wherein the donor fluorescent protein is CFP or a mutant thereof, and the acceptor fluorescent protein is YFP or a mutant thereof.

4. The fluorescent indicator according to any of claims 1 to 3 wherein the donor fluorescent protein and/or the acceptor fluorescent protein are circularly permuted fluorescent proteins, which are obtained by substituting the amino acid sequence on the N-terminal side with the amino acid sequence on the C-terminal side with respect to amino acid residues positioned in a β turn in the amino acid sequence of a wild-type fluorescent protein or a mutant protein thereof.

5. The fluorescent indicator according to claim 4 wherein the amino acid residues positioned in the β turn are amino acid residues which are positioned such that the

dynamic range of the fluorescence of a fluorescent protein can be increased.

6. The fluorescent indicator according to any of claims 1 to 5 wherein the acceptor fluorescent protein is a circularly permuted version of the fluorescent protein Venus.

7. The fluorescent indicator according to claim 6 wherein the circularly permuted versions of Venus are cp49Venus, cp157Venus, cp173Venus, cp195Venus, or cp229Venus.

8. The fluorescent indicator according to any of claims 1 to 7 which further comprises a target peptide component and a linker component,

wherein the target sequence of the analytical substance further comprises a peptide-binding domain for allowing the target peptide component to bind thereto,

wherein the linker component allows the target sequence of the analytical substance to covalently bind to the target peptide component, and the target sequence and the target peptide component covalently bind to either the acceptor fluorescent molecular component or the donor fluorescent molecular component, and

wherein the analytical substance binding to the target sequence induces a change in the relative positions or orientations of the target peptide component and the peptide-binding domain, and the relative positions or orientations of the donor molecular component and acceptor molecular component are then changed, thereby changing the efficiency of fluorescence resonance energy transfer (FRET).

9. The fluorescent indicator according to any of claims 1 to 8 wherein the target sequence is calmodulin, cGMP-dependent protein kinase, a steroid hormone receptor, a ligand-binding domain of a steroid hormone receptor, protein kinase C, inositol-1,4,5-triphosphate receptor, or recobelin.

10. The fluorescent indicator according to claim 9 wherein the target sequence is calmodulin.

11. The fluorescent indicator according to claim 8 wherein the target peptide component is skeletal muscle myosin light chain kinase (skMLCKp), smooth muscle

light chain kinase (smMLCK), calmodulin kinase II (CaMKII), caldesmon, calspermine, phosphofructokinase, calcineurin, phosphorylase kinase, Ca^{2+}-ATPase, 59 Kda phosphodiesterase (PDE), 60 Kda phosphodiesterase (PDE), nitric oxide synthase, type I adenylyl cyclase, *Bordetella pertussis* adenylyl cyclase, neuromodulin, spectrin, myristoylated alanine-rich C kinase substrate (MARCKS), MacMARCKS(F52), b-Adducin, heat shock protein HSP90a, human immunodeficiency virus envelope glycoprotein 160 (HIV-1 gp160), brush-boarder myosin heavy chain-I (BBMHBI), dilute myosin heavy chain (MHC), mastoparan, melittin, glucagon, secretin, vasoactive intestinal peptide (VIP), gastrin inhibitory peptide (GIP), or a calmodulin-binding domain of calmodulin-binding peptide-2 (Model peptide CBP2).

12. The fluorescent indicator according to claim 8 wherein the linker component is a peptide component consisting of 1 to 30 amino acid residues.

13. The fluorescent indicator according to any of claims 1 to 12 which further comprises a localizing sequence.

14. The fluorescent indicator according to any of claims 1 to 13 wherein the localizing sequence is a nucleus-localizing sequence, an endoplasmic reticulum-localizing sequence, a peroxisome-localizing sequence, a mitochondrion-localizing sequence, a Goldi apparatus-localizing sequence, or a cell membrane-localizing sequence.

15. A fluorescent indicator having the amino acid sequence shown in any one of SEQ ID NOS: 42, 43, 44, 45, and 46.

16. A method for detecting or measuring an analytical substance contained in a sample, which comprises:

(1) a step of allowing a sample to come into contact with the fluorescent indicator of any of claims 1 to 15;

(2) a step of exciting a donor component; and

(3) a step of measuring the level of fluorescence resonance energy transfer in the sample

that corresponds to the concentration or activity of the analytical substance contained in the sample.

17. The method according to claim 16 wherein the sample is a living cell, and said contact step includes incorporation of the fluorescent indicator into such a living cell.

18. The method according to claim 17 wherein the step of incorporating the fluorescent indicator into a cell comprises transfection of the cell with an expression vector which contains an expression regulatory sequence that is functionally ligated to a nucleic acid sequence encoding the expression of the fluorescent indicator.

19. A nucleic acid encoding the fluorescent indicator of any of claims 1 to 15.

20. An expression vector containing the nucleic acid of claim 19.

21. A transformant having the nucleic acid of claim 19 or the expression vector of claim 20.